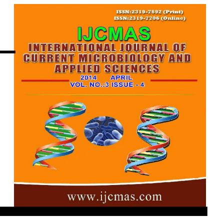
Int.J.Curr.Microbiol.App.Sci (2014) 3(5): 881-889



International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 5 (2014) pp. 881-889**
http://www.ijcmas.com

Original Research Article

Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in *Catharanthus roseus*

R.Mangaiyarkarasi[1*], M.Girija[2] and S.Gnanamurthy[2]

[1]CKN College, Thiruvalluvar University , Tamil Nadu, India
[2]Department of Botany, Annamalai University, Tamil Nadu, India
Corresponding author

A B S T R A C T

Keywords

Mutagens, Effectiveness, Efficiency, Gamma rays, EMS, *Catharanthus roseus.*

Mutagenic effectiveness and efficiency of gamma rays and EMS were studied in the genotype of *Catharanthus roseus*. The plant *Catharanthus roseus* is a perennial and self-pollinated plant, is of immense medicinal value due to antipotential activities of its leaf alkaloids. A mutation breeding programme was carried out to design a physiologically and chemically efficient plant type with increased production of secondary metabolites. The mutagenic treatments seeds were tested for lethal dose 50 percent for all mutagens, separately and the dose at which 50 percent of the seed germination was considered as LD_{50} values. Gamma rays and EMS are produced a high frequency as well as a wide spectrum of mutation. The frequency of mutation was more in EMS than gamma rays. The mutagenic effectiveness and efficiency was calculated based on biological damage. In M_1 generation based on seed lethality (L) and seedling injury (I) and M_2 generation was carefully screened for various chlorophyll and viable mutations. Mutagenic effectiveness and efficiency increased with the decreased in dose or concentration. In the present study EMS was proved to be more effective and efficient in causing mutations as compared to gamma rays treatments.

Introduction

Catharanthus roseus (L.) G. Don (Periwinkle), a perennial tropical plant of family Apocynaceae, produces a high number of monoterpenoid indole alkaloids of which two dimeric alkaloids, vincristine and vinblastine are clinically useful oncolytic drugs (Cordell, 1980). It is cultivated as an ornamental plant almost throughout the tropical and subtropical world. It is abundantly naturalized in many regions, particularly in arid coastal locations. The herb has been used for centuries to treat a variety of ailments and was a favorite ingredient of magical charms in the middle ages. The Latin name for this herb is *Catharanthus roseus*, but it was formerly classified as vinca rosea, and is still called by that name in some of the herbal literature. These two bis-indole alkaloids occur in trace amounts

in leaves and are products of oxidative coupling of catharanthine and vindoline.
Periwinkle is also mentioned in folk-lore remedies for treatment of diabetes (Githens, 1949), malaria (Duke, 1985), dysentery (Virmani et al., 1978), insect bite (Sukumar and Osmani 1981), kidney disorder (Morton, 1977), irregular menstrual cycle (Perry, 1980), and skin infections (Virmani et al., 1978). The pharmacological and therapeutic value of this plant has promoted intensive research for the development of its improved cultivars. Among various improvement methods, mutation breeding is one of the promising approaches, for development of such improved ideochemovars (Swaminathan, 1972).

Periwinkle has been used as an effective astringent that can be used orally or topically. It is mainly used to treat excessive menstrual bleeding but may also be a helpful choice in cases of colitis, diarrhea, bleeding gums, nosebleeds, sore throats, and mouth ulcers. Periwinkle should not be used by people with low blood pressure or constipation. Due to lack of sufficient medical study, periwinkle should be used with caution in children, women who are pregnant or breast-feeding, and people with liver or kidney disease. Hence mutagenesis in *Catharanthus roseus* was attempted to induce the useful mutations and a study was undertaken for evaluation of morphological parameters and the antibacterial activity of the aqueous and mutant and control plants.
Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetics, biotechnology and molecular biology etc. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya *et al.,* 2007) and an efficient means supplementing existing germplasm for cultivar improvement in breeding program's (Dubinin, 1961).

Shah *et al.*, (2008) reported that mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants. Among the chemical mutagens, EMS is reported to be the most effective and powerful mutagen (Minocha & Arnason 1962). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissue (Gunckel & Sparrow 1961). Khatri *et al.*, (2005) reported that gamma rays and EMS could be fruitfully applied to develop new varieties with high yield and other improved organic traits. EMS induce a high rate of mutations in both micro and higher organisms (Freese, 1963) and sometimes the mutation frequencies exceed those obtained by radiation (Goud, 1967).

The usefulness of a mutagens in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagens), but also on its mutagenic efficiency (mutation in relation to undesirable changes like sterility, lethality, injury etc.). The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations (Solanki & Sharma, 1994) and (Mahabatra, 1983). The present investigation was undertaken to study the frequency and spectrum of macro mutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays and EMS treatments were undertaken in *Catharanthus roseus.*

Materials and Methods

The dry and dormant seeds of the periwinkle *(Catharanthus roseus*) were treated with gamma rays, EMS and their combination treatments were used in the present study. Healthy seeds packed in moist germination paper were selected for each treatment in the gamma chamber at 30, 40, 50, 60 & 70 KR doses of gamma rays in ^{60}CO gamma source (irradiation source capacity to release 3000 Ci delivery 7200 r/min). The gamma irradiation was carried out at sugarcane breeding institute (ICAR), Coimbatore, India. For EMS treatment, the seeds were presoaked in distilled water in 3 hours. The presoaked seeds were treated with 30, 40, 50, 60 & 70mM freshly prepared solution for 3 hours. After the EMS treatment, the treated seeds were washed thoroughly for 1h in running tap water to terminate the residual effect of the mutagenic chemicals. After the completion of the treatment the treated seeds were sown immediately in the field along with their respective controls to raise the M_1 generation in a randomized block design with three replications.

The seedling height reduction (I) in different M_1 generation was studied following Nilan *et al.,* (1965), Sharma (1990) and velu *et al.,* (2007). The plant survival (L) was computed as the percentage of plants surviving till maturity. The biological damage (lethality/ injury) was computed as the reduction in plant survival and plant height. At maturity all the surviving M_1 fertile plants were harvested separately and seeds were sown in the next season in plant progeny rows to raise M_2 generation. The respective control and treatment progenies were screened several times for morphological mutations throughout the crop duration. Different kinds of chlorophyll mutants (Xantha, chlorina and albina) were scored from emergence till the age of four week in M_2 generation by using modified classification of Lampretcht (1960) and Kharkwal (1998). Mutation frequency was calculated as percentage of mutated M_2 progenies for both chlorophyll and morphological mutations in each treatment. The Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al.,* (1965).

Mutagenic effectiveness (Physical mutagens) = Mf×100/krad.

Mutagenic effectiveness (Chemical mutagens) = Mf×100/c×t.

Mf= chlorophyll / viable mutants per100 M_2 plants

t=Period of treatment with chemical mutagen in hours

C=Concentration of mutagen in mM in percent

Krad = dose of mutagenic radiation in kilo rad

L=Percentage of lethality (or) survival reduction

I=Percentage of injury (or) reduction in seedling size.

Results and Discussion

LD_{50} value was calculated on the basis of 50 percent reduction of germination seeds count on 10th day. The present investigation exhibited that the germination percentage of *Catharanthus roseus* decreased with the increase in the

Dose or concentration of the mutagens were used to find out the LD_{50} values for further studies. It was estimated that using 50% reduction in seed germination observed at 50KR gamma rays and 50mM of EMS. Similar results were observed in cowpea, mungbean and Bengal gram as reported by Palaniswamy (1975), Louis kadambavana sundaram (1973) and Vadivelu (1979) respectively. The frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measure of genetic effect in mutagen (Nilan & Konzak, 1961 and Gautam *et al.*, 1998). The mutation frequency showed a decrease with increase in the dose or concentration of mutagens. In the present investigation, the maximum chlorophyll and viable mutation frequency observed at 50mM of EMS (2.72). While the minimum chlorophyll and viable mutation frequency was observed at 30KR of gamma rays (1.) (Table-2).

Chlorophyll Mutant

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various pulse crops by several workers including Gautam *et al*., (1992). The frequency of chlorophyll mutants in M_2 generation is mainly used as a dependable measure of genetic effects in mutagens (Nilan and Konzak, 1961).

On the seedling basis of M_2 generation, progressive increase in the frequency of chlorophyll mutation was observed with increase in all mutagenic dose or concentration. Albino mutant leaves were white in colour, due to absence of all pigment. This was leaded to the death of the plants at 10-15 days after germination. Xantha mutant leaves turned yellow in colour due to the absence of xanthophylls. Chlorina mutants showed all chlorophyll pigments are absent in treated plants compared to control. Young leaves were pale green in during maturity time. One or two mutants were observed at all mutagenic treatments.

Keeping in view, it was observed in M2 generation that EMS was more pronounced in inducing chlorophyll mutations than gamma rays (shah et al., 2006) and among the spectrum, the viridis (less drastic mutation) was more than that of albina (extreme mutation) as categorized by Westergaard (1960).

Viable Mutant

In the present investigation, some of the morphological (viable) mutants were observed in M_2 generation with different dose or concentration of gamma rays and EMS treatment an increase in the number of viable mutants were realized in the present study. 50mM of EMS produced more number of viable mutants than gamma rays treatments.

Tall and dwarf mutants were observed in different mutagenic treatments. Among the dose or concentration of maximum number of mutants were recorded at 50KR of gamma rays and 50mM of EMS. Similar mutants were observed by Kumar and Dubey (1998) in *Lathyrus sativus*, Ramesh and Seetharami Reddi (2002) in rice. The leaf mutant such as long leaf was observed in different mutagenic treatments. Among the dose or concentration maximum number of leaf mutants was recorded at 50mM of EMS treatments. Similar mutants were observed by Sengupta & Datta (2005) in sesame.

Mutagenic Effectiveness and Efficiency

Effectiveness means the rate of mutation induction as dependent upon the mutagenic does and efficiency refers to the mutation rate in mutation to the various biological effects usually a measure of damage (Nilan *et al.,* 1965). In general the effectiveness decreased with increasing dose or concentration. With increasing doses of EMS or Gamma rays the values obtained for all the biological criteria for M_1 generation were decreased. The reduction in biological criteria (Plant height & Survival) may be attributed to a drop in the auxin level (Gordon & Webber, 1955), inhibition of auxin synthesis (Skoog, 1935). EMS was found to be more effective than gamma rays and combined treatments in inducing mutation. The maximum mutagenic effectiveness was observed at 50KR of gamma rays (5.0) and the minimum mutagenic effectiveness was observed at 30mM of EMS treatments (0.52). Similar results were recorded by Solanki (2005) in lentil; Yadava *et al*., (2003) in kodo miilet.

The mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen (Konzak *et al.,* 1965). Efficient mutagens and their treatments are indispensable for the cost-effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding programme.

On the basis of lethality, the highest mutagenic efficiency was recorded at 50KR of gamma rays (5.61) and the lowest mutagenic efficiency was observed at 70KR of gamma rays (0.99). On the basis of injury, the maximum mutagenic efficiency was observed at 50KR of gamma rays (7.24). The minimum mutagenic efficiency was observed at 70 KR of gamma rays (1.34). In general the mutagenic treatment 50KR gamma rays were found to be highly efficient to induce chlorophyll and viable mutants (Table-3). Similar results recorded by Jayakumar and Selvaraj (2003) in sunflower, Yadava *et al.*, (2003) in kodo-millet and Jabee and Ansari (2005) in chickpea.

The treated C. roseus plants showed an increase in plant height when compared to control. The main reason for increase in plant height is due to the increased root growth under mutagenic treatments. The mutagenic treatments with gamma rays and EMS induced GA increased the whole plant of C. roseus. The increase in plant height was significant when compared to control. It can be interfered that the higher chlorophyll content in the leaves, leading to higher photosynthesis might have increased plant height in the mutagen treated C. roseus. It was apparent that treating plants increased height of the plant over the control, which may be attributed to the growth promotion effect of GA3 in stimulating and accelerating cell division, increasing cell elongation and enlargement or both (Jaleel et al., 2007) which in turn increased the plant height of the plants. *Catharanthus roseus* produces widely used alkaloids such as the anticancer drugs vinblastine and vincristine, as well as the antihypertensive compounds ajmalicine and serpentine. Catharanthine, tabersonine, lochnericine and horhammericine are other indole alkaloids found in C. roseus. In conclusion, EMS was proved to be more effective and efficient than gamma rays.

Table.1 Effect of Gamma rays and Ems on Seed Germination and Plant Height in *Catharanthus roseus*

Treatment Dose/Conc.		Seed germination (%) 30th day	Percent of reduction over control	Plant height (cm) 30th day	Percent of reduction over control
Control		95.00	100.00	28.00	100.00
Gamma rays (KR)	30KR	73.00	23.15	25.73	8.10
	40KR	65.33	31.23	22.26	20.50
	50KR	52.66	44.56	18.34	34.50
	60KR	34.66	63.51	15.53	45.25
	70KR	22.33	76.49	12.16	56.57
EMS (Conc. mM)	30mM	71.00	25.26	23.26	
	40mM	62.33	34.38	21.34	16.92
	50mM	48.66	48.77	15.78	23.78
	60mM	32.66	65.62	13.67	43.64
	70mM	20.33	78.60	10.58	51.17
					62.21

Table.2 Frequency of Chlorophyll and Viable Mutants In M_2 Generation of *Catharanthus roseus*

Treatment Dose/Conc.		Total plants studied in M_2 generation	Total plant segregated in M_2 generation	Mutation frequency
Gamma rays (KR	30KR	720	3	0.41
	40KR	580	5	0.86
	50KR	400	10	2.50
	60KR	275	4	1.45
	70KR	130	1	0.76
EMS (Conc. mM)	30mM	775	4	0.51
	40mM	630	6	0.91
	50mM	550	15	2.72
	60mM	345	8	2.31
	70mM	270	3	1.11

Table.3 Mutagenic Effectiveness and Efficiency in M_2 Generation of *Catharanthus roseus*

Treatment	Dose/Conc.	Survival Reduction (Lethality) (%)	Height Reduction (Injury) (%)	Mutation Frequency	Effectiveness $\frac{M \times 100}{KR\ (or)\ C \times T}$	Efficiency $\frac{M \times 100}{L}$	Efficiency $\frac{M \times 100}{I}$
Gamma rays (KR)	30KR	23.15	8.10	0.41	1.36	1.77	5.06
	40KR	31.23	20.50	0.86	2.15	2.75	4.19
	50KR	44.56	34.50	2.50	5.0	5.61	7.24
	60KR	63.51	45.25	1.45	2.41	2.28	3.20
	70KR	76.49	56.57	0.76	1.08	0.99	1.34
EMS (Conc. mM)	30mM	25.26	16.92	0.51	0.56	2.01	3.01
	40mM	34.38	23.78	0.91	0.75	2.68	3.86
	50mM	48.77	43.64	2.72	1.81	5.57	6.23
	60mM	65.62	51.17	2.31	1.28	3.52	4.51
	70mM	78.60	62.21	1.11	0.52	2.28	1.78

References

Acharya SN, Thomas JE, Basu SK .2007. Improvement in the medicinal and nutritional properties of fenugreek (*Trigonella foenum-graecum* L.). In: Acharya SN, Thomas JE (eds) Advances in medicinal plant research, Research Signpost, Trivandrum, Kerala, India.

Cordell GA. The botanical, chemical biosynthesis and pharmacological aspect of *Catharanthus roseus* (L.) G. Don (Apocynaceae), 1980. In: Woo WS, Han BH, editors. Recent Advances in National Product Research. Seoul National University Seoul: University Press; pp. 65–72.

Dubinin NP. 1961. Problems of radiation genetics. Oliver & Boyd, London.

Duke., J. A. 1985. Handbook of Medicinal Herbs: Catharanthus roseus. Boca Raton, FL: CRC Press.

Freese, E. 1963. Molecular mechanism of mutations. In: Molecular genetics, Part I (Ed. J.H. Taylor): 207-269.

Gautam, A.S., K.C. Sood and A.K. Richarria, 1992. Mutagenic effectiveness and efficiency of gamma rays, ethylmethane sulphonate and their synergistic effects in blackgram (*Vigna mungo* L.). *Cytologia*, 57: 85-89.

Gautam, A.S., K.C. Sood and R.K. Mittal, 1998. Mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in rajmash (*Phaseolus vulgaris* L.). *Legume Res.*, 21(3&4): 217-220.

Githens TS. 1949. Drug Plant of Africa. Philadelphia: University of Pennsylvania Press.

Gordon, S.A. and R.P. Webber 1955. Studies on the mechanism of phytohormone

Goud, J.V. 1967. Induced mutations in bread wheat. Indian J. Genet. 27: 40-55.

Gunckel, J.E. and A.H. Sparrow 1961. Ionizing radiation: Biochemical, Physiological and Morphological aspects of their effects on plants. In: *Encycl. Plant Physiol.* (ed.) W. Ruhland. XVI: pp. 555-611, Springer-verlag, Berlin

Jabee, F. and M.Y.K. Ansari, 2005. Mutagenic effectiveness and efficiency of hydrazine sulphate (HS) in indusing cytomorphological mutation in *Cicer arietinum* L. var. K. 850. *J.Cytol. Genet.*, 6(2): 161-166.

Jaleel, C.A., R. Gopi, P. Manivannan, B. Sankar, A. Kishorekumar and R. Panneerselvam, 2007. Antioxidant potentials and ajmalicine accumulation in Catharanthus roseus after treatment with giberellic acid. Colloids and Surfaces B: Biointerfaces, 60(2): 195-200.

Jayakumar, S. and R. Selvaraj, 2003. Mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in sunflower (*Helianthus annuus* L.). *Madras Agric. J.*, 90(7-9): 574-576.

Kharakwal, M.C. 1998. Induced mutations for improvement of protein in chickpea (*Cicer arietinum* L.). *Indian J. Genet.*, 58: 61-68.

Khatri, A., I.A. Khan, M.A. Siddiqui, S. Raza and G.S. Nizamani. 2005. Evaluation of high yielding mutants of *Brassica juncea* cv. S-9 developed through gamma rays and EMS. *Pak. J. Bot.*, 37(2): 279-284.

Konzak, C.F., R.A. Nilan, J. Wagner and R.J. Foster, 1965. Efficient chemical mutagenesis. The use of induced mutations in plant breeding Rept. FAO/IAEA Tech. Meet. Rome.

Kumar, S. and D.K. Dubey, 1998. Induced morphological mutations in *Lathyrus sativus*. *J. Cytol. Genet.*, 33(2): 131-137.

Lamprecht, H. 1960. Über Blattfarben von Phanerogamen. Klassifikation, Terminologie und Gensymbole von chlorophyll und anderen Farbmutanten. *Agri. Hort. Gen.*,18:135-168.

Lois, I.H. and Kadambavasundaram, M. 1973. Mutation breeding in cowpea. An evaluation of selection methods in M1 generation. Madras Agric.J.60: 1361-1368.

Mahabatra, B.K., 1983. Studies on comparative spectrum and frequency of induced genetic variability in green gram [*Vigna radiata* (L.) Wilczek]. Ph.D. Thesis, IARI, New Delhi.

Minocha, J.L. and T.J. Arnason.1962. Mutagenic effectiveness of ethyl methane sulfonate in barley. *Nature*, 196: 499.

Morton JF, 1977. Editor, Major Medicinal Plants, Botany Culture and Uses. Springfield, IL: Charles C. Thomas Publishing Company; pp. 236–41.

Nilan, R.A. and C.F. Konzak, 1961. Increasing the efficiency of mutation breeding. Mutation and plant breeding. *NAS-NRC,* 891: 437-460.

Nilan, R.A., C.F. Fonzak J. Wagner & R.R. Legault. 1965. Effectiveness and efficiency of radiation for inducing genetic and cytogenetic changes. Rad. Bot., 5: 71-89.

Palaniswamy, G.A.1975. Investigation on the induction of mutations in cowpea (Vigna sinensis L. Savi) M.Sc Thesis, Tamil Nadu Agric. Univ. Coimbatore, India.

Perry LM. 1980. Medicinal Plants of East and South-east Asia. Cambridge: Met Press.

Ramesh, D.V. and T.V.V.S. Reddi, 2002. Induced morphological variability among three genotypes of rice. *J. Cytol. Genet.*, 3(2): 115-120.

Sengupta, S. and A.K. Datta, 2005. Induced narrow leaf mutant of Sesame (*Sesamum indicum* L.).Indian J. Genet., 65(1): 59-60.

Shah, T.M., J.I. Mirza, M.A. Haq and B.M. Atta. 2008. Induced genetic variability in chickpea (*Cicer arietinum L*.). II. Comparative mutagenic effectiveness and efficincy of physical and chemical mutagens. Pak. J. Bot., 40 (2): 605-613.

Shah, T.M., J.I. Mirza, M.A. Haq and B.M. Atta. 2006. Induced genetic variability in chickpea (*Cicer arietinum L*.). I. Frequency and spectrum of chlorophyll mutations. *Pak. J. Bot.*, 38(4): 1217-1226.

Sharma, S.K., 1990. Mutagenic effectiveness and efficiency in macrosperma lentil. Cytologia, 55: 243-247.

Skoog, F. 1935. The effect of X-irradiation on auxin and plant growth. *J. Cellular Comp. Physiol.*,7: 227-270.

Solanki, I.S. and B. Sharma, 1994. Mutagenic effectiveness and efficiency of gamma rays, ethylene imine and N-nitroso-N-ethyl urea in macrosperma lentil (*Lens culinaris* Medik.). *Indian J. Genet.*, 54(1): 72-76.

Solanki, I.S., 2005. Isolation of macromutations and mutagenic effectiveness and efficiency in lentil (*Lens culinaris* Medik.). *Indian J. Genet.*, 65(4): 264-268.

Sukumar K, Osmani Z. 1981. Insect sterilants from *Catharanthus roseus*. Current Sci (India); 50:552–3.

Swaminathan., M.S.1977. Mutational reconstruction of plant ideotypes In: Induced mutation and Plant improvement. Vienna: IAEA; pp. 155–77.

Vadivelu, K.K. 1979. Studies on induced mutations in bengal gram (Cicer arietinum L.) Ph.D Thesis, Tamil Nadu Agric. Univ. Coimbatore, India

Velu, S., L. Mullainathan., D. Arulbalachandran., D. Dhanavel and R.Poonguzhali, 2007. Effectiveness and Efficiency of gamma rays and EMS on cluster bean (*Cyamopsis tetragonoloba*(L.) Taub.). *Crop Res*.34 (1, 2&3): 249-251.

Virmani OP, Srivastava GN, Singh P. 1978. *Catharanthus roseus*. The Tropical Periwinkle. Indian Drugs, 15:231–52.

Westergaard, M. 1960. A discussion of mutagen specificity, Chemis che Mutagenese. *Erwin Baur Ged Vorl*., 116-121.

Yadava, H.S., A.N. Tikle and D.V. Bhagat, 2003. Effect of induced mutation through gamma rays on growth and yield parameters of kodo-millet. *J. Soil and Crops*, 13(1): 25-28.